# THE AXIAL GRADIENTS IN HYDROZOA. VIII. RESPIRATORY DIFFERENCES ALONG THE AXIS IN TUBULARIA WITH SOME REMARKS ON REGENERATION RATE.

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## AXIAL DIFFERENCES IN OXYGEN CONSUMPTION.

The experiments recorded in this section were performed at Swan's Island, Maine, in August, 1924. This island is one of a group off the coast of Maine, opposite Rockford, and is directly south of Mt. Desert Island. My visit to Swan's Island was occasioned through information received from the Anglers Company (now the Denoyer-Geppert Company) that *Tubularia* flourishes in that locality during the summer months, at which season, as is well known, it is in poor condition at Woods Hole. The company further kindly invited me to make use of their collecting station and equipment located on Swan's Island. I am greatly indebted to the company in all of these matters and particularly to Mr. Philip Turner, a member of the firm, for assistance in collecting *Tubularia*.

The collecting station at which these experiments were performed is situated in the town of Swan's Island, which town is located at the head of a large harbor and is provided with the usual wharves, pilings, etc. *Tubularia* was found in large quantities on the pilings of the steamer wharf and is apparently restricted to this one habitat. Members of the Anglers Company informed me that they had never seen it in any other situation nor on any other of the wharves or pilings. Large quantities of the hydroid were readily obtained at low tide from the piles supporting the wharf.

The *Tubularia* occurring at Swan's Island does not seem to be identical with the *T. crocea* of Woods Hole. The stems are much more elongated and branching less frequent. These character-

istics may, however, result from the low temperature of the water, which did not, on the warmest summer days, exceed 15° C. The species is very favorable for experimental work, owing to the long unbranched stems and their freedom from other growths.

For the oxygen consumption large colonies were collected and from these a number of long straight clean unbranched stems were selected. They were carefully examined under the microscope to determine that they were free from diatoms or other organisms. In some cases a few such organisms were seen growing on the perisarc but it was found that these could be removed by gently brushing the stem with a camel's hair brush. The hydranth and upper two or three millimeters and the basal part of the stem were then cut off, leaving a clean stem twenty to thirty millimeters long. This was then cut into two pieces, the basal piece generally taken a little longer than the apical piece to compensate for its smaller diameter. Six to eight such pieces were used in each experiment and an attempt was made to select for each experiment stems of similar diameter. All stems used in any one experiment came from the same colony.

The method of determining the oxygen consumption was the same as previously employed in a similar study on *Grantia* (Hyman, '25). For details this paper should be consulted. Briefly small tubes detachable into two sections are employed. At the end of the experiment the pieces of stem are brought by gravity into one section, which is then removed; the other section is analyzed for oxygen content. In each experiment four tubes are employed: one containing the six to eight apical pieces of stem; one containing the basal pieces of the same stems; and two water blanks.

To compare the rate of oxygen consumption of two objects, it is necessary to know the quantity of protoplasm in each. Owing to the lack of a balance at Swan's Island, the weights of the pieces could not be determined. There would be some difficulty in determining the weight, owing to the presence of the lifeless perisarc around the stems; but some method could probably be devised to eliminate this difficulty. Under the circumstances, however, I was compelled to use the volume of the pieces as a standard of comparison. After each experiment the diameters of the stems were determined under the compound microscope with an ocular micrometer. However uniform a stem may appear to the eye naturally under the microscope considerable variations in diameter are perceived. Some fifteen to thirty measurements of the diameter of the cœnosarc were made at frequent intervals along each stem and these were averaged. The length of the piece was measured on a millimeter rule. From the average diameter and the length the volume of the piece was calculated assuming it to be a cylinder. No correction was made for the central cavity. The volumes of all of the apical pieces in any one experiment were added together and the volumes of the basal pieces similarly; and when the oxygen consumed is divided by these volumes, the quotients can be used to compare the rates of oxygen consumption of apical and basal pieces.

There is no doubt that considerable error is involved in such determinations of the volume of cœnosarc in the pieces. It does not seem to me, however, that the weight of the cœnosarc could be determined any more accurately. All determinations of the rate of respiratory metabolism are necessarily erroneous since there is no known way of discovering the actual quantity of respiring protoplasm in an organism. The consistent results which I have obtained in *Tubularia*, the definite relation noted between level and oxygen consumption, and diameter and oxygen consumption, indicate that the experiments are sufficiently accurate to render the conclusions acceptable.

Nine experiments were performed. The results are presented in Table I. The first column of figures in this table gives the oxygen content in cubic centimeters of the water in the tube at the beginning of each experiment; the second column the oxygen content in the tube at the end; and in the third column is given the difference between the first and second columns, or the oxygen consumed by the pieces. The data are presented in this way for the sake of simplicity; they are not actually obtained in this form as the original oxygen content of the tubes containing the pieces has to be calculated from the blanks. The differences in oxygen content of the two tubes in each experiment at the start are due simply to differences in the volumes of the tubes; for the same water is used in both tubes in each experiment. The fourth column of figures gives the total volume of the pieces in cubic millimeters, the fifth column the average diameter of the pieces, and the sixth column the oxygen consumed per cubic millimeter in the time occupied by the experiment. Each experiment lasted four hours. In the first experiment six

## TABLE I.

Showing the Cubic Centimeters of Oxygen Consumed per Cubic Millimeter of Volume in Four Hours by Apical and Basal Pieces of the Stem of *Tubularia*.

No. of Exp.	Level of Pieces	O2 Con- tent at Start, cc.	O2 Con- tent at End, cc.	O2 Con- sumed, cc.	Vol. of Cœno- sarc, cu. mm.	Aver. Diam., mm.	O2 Con- sumed per cu. mm. in Four Hrs.
4	Apical	.070	.061	.009	32.97	.63	.00027
	Basal	.060	.054	.006	30.84	.59	.00019
5	Apical	.068	.060	.008	24.71	•55	.00032
	Basal	.061	.055	.006	21.90	•53	.00027
3	Apical	.065	.055	.010.	20.51	.58	.00048
	Basal	.060	.052	800.	18.68	.46	.00042
6	Apical	.065	.055	.010	20.34	•53	.00049
	Basal	.065	.057	.008	19.61	•51	.00040
7	Apical	.077	.067	.010	18.39	.50	.00054
	Basal	.067	.060	.007	19.25	.48	.00036
9	Apical	.072	.057	.015	20.25	.49	.00074
	Basal	.068	.057	.011	21.76	.51	.00050
8	Apical	.076	.061	.015	17.02	.46	.00088
	Basal	.071	.061	.010	15.08	•44	.00066
I	Apical Basal	.068 .058	.058 .052	.010. 600.	11.48 11.10	.43 .41	.00087
2	Apical	.067	.056	.011	13.52	.38	.00081
	Basal	.063	.055	800.	14.59	.35	.00054

Duration of all experiments, four hours.

pieces of stem were used; in the other experiments eight pieces. The temperature was 20° C. in experiments I to 3 and 16° C. in experiments 4 to 9. The volumes of oxygen as presented in the table have been corrected for these temperatures. The nine experiments are arranged in the table in the order of the diameters of the stems used, largest first, smallest last. As already stated,

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the six or eight stems in each experiment were selected so as to be of similar diameters.

The following generalizations may be drawn from the data given in Table I.:

1. In all cases the rate of oxygen consumption per unit volume is higher in the apical than in the basal pieces. This result is in harmony with differences in rate of regeneration and in electrical potential which exist along the stem of *Tubularia* (cf. Hyman, '20).

2. There is an inverse relation between the diameter of the stems used and the rate of oxygen consumption, the rate being higher the smaller the diameter of the stems. This agrees with the general law that in animals respiratory rate is inversely proportional to size.

3. In general it appears that the respiratory differences between apical and basal pieces are greater the smaller the diameter of the stem. This indicates that the respiratory gradient is steeper the more slender the stem.

These conclusions are considered further in the discussion.

## EXPERIMENTS ON REGENERATION.

Data on certain questions regarding regeneration which were obtained on *Tubularia marina* at Pacific Grove, California, in 1922, on *Tubularia crocea* at Woods Hole, Massachusetts, in 1924, and on *Tubularia* sp. at Swan's Island, Maine, in 1924 are herein presented. Owing to the large amount of work that has already been done on the regeneration of *Tubularia*, it does not seem necessary to present these data in detail. For the most part general statements will be made.

The expression "rate of regeneration" is defined and used to mean the time which elapses between the act of cutting and the attainment of a condition of equilibrium. Since in pieces of *Tubularia* of the size employed complete regeneration of a hydranth at the apical end of the piece always occurs, a condition of equilibrium is here synonymous with the completion of a hydranth. Practically, however, it is difficult or impossible to determine the exact time at which the regenerated hydranth is complete. As is well known, the new hydranth forms within the perisarc and the time of completion of its formation in situ would furnish the most accurate measure of rate of regeneration if such time could be determined. For practical purposes one generally notes the time at which the finished hydranth emerges from the top of the old perisarc. There is no doubt some error in such a procedure for in some cases at least such emergence may be greatly delayed by irregularities in the perisarc through which the hydranth must force its way. However, no better criterion of the time of completion of regeneration has been suggested by anyone and the time occupied by the regeneration process will here be used to signify the time between cutting and the emergence of the completed hydranth.

In all regeneration experiments straight healthy unbranched stems were employed. These were invariably cut as follows. The hydranth and upper millimeter or two and the basal portion of the stem were removed and discarded. The remaining stem was then cut as desired.

The regeneration of only the oral hydranth—*i.e.*, the hydranth which forms at the apical end of each piece—was studied. Unless specifically stated otherwise, all statements refer to this hydranth only. No study was made of the regeneration of aboral hydranths.

1. Rate of Regeneration of Halves of the Stem.—In such experiments the stem is divided into halves and the time between cutting and emergence of oral hydranths noted for apical and basal halves. As above stated the observations refer only to regenerated oral hydranths. In a previous paper (Hyman, '20) I presented a considerable mass of data on this matter using *Tubularia crocea*. These experiments showed that the time between cutting and emergence of oral hydranths is markedly shorter in apical than in basal halves. Since writing that paper the same result has been obtained with *T. marina* and with the Maine species. The result on *T. crocea* was also again verified. In Table II, I present a typical experiment on *T. marina*.

2. Rate of Regeneration of Thirds of the Stem.—A few experiments on this point were performed on T. marina and on the Maine species. The result was found to be different in the two species. In T. marina, the apical third regenerates first, the middle third next, and the basal third last, the differences between the three pieces being well-marked. The basal third in *T. marina* is generally much behind the other pieces. A typical experiment on *T. marina* is given in Table II. On the contrary in the Maine species, the time between cutting and emergence of hydranths is about the same for the middle and basal thirds, the apical third being in advance as usual. I have, however, but five sets of pieces in which the time of emergence of the oral hydranths was exactly determined in all three pieces. These times are as follows, apical third first, middle third next, basal last: 39, 45, 46 hours; 41, 52, 52 hours; 42, 53, 53 hours; 43, 50, 53 hours; and 41, 46, 46 hours. These pieces were 8 to 10 mm. long.

## TABLE II.

REGENERATION RATE OF HALVES AND THIRDS OF THE STEM OF Tubularia marina.

The first column gives the hours elapsed between cutting and emergence of the oral hydranths; and the other columns the number of hydranths emerged at the hours indicated. Length of halves, 3-7 mm.; length of thirds, 5-8 mm.

Hours Since	Halves.		Thirds.		
Cutting.	Apical.	Basal.	Apical.	Middle.	Basal.
29	I	0	I	0	0
30	3	0	1	0	0
31	6	1	I	0	0
32.5	9	2	3	0	0
33.5	12	4	6	0	0
34.5	1.4	9	8	0	0
35.5	17	11	9	2	0
37	18	14	10	3	0
39	19	16	IO	6	0
41.5		18	10	6	0
43		19	10	9	1
44.5			01	9	3
45.5			10	10	-1
40.5			I 1	10	-1
48.5				10	0
50.5		1		1.1	6
52.5					8
54.5					9
56.5					11

It thus appears that the physiological differences along the stem which are responsible for the differences in regeneration rate at different levels extend further down the stem in *T. marina* than in either of the Atlantic coast species. Banus ('18)

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had previously shown a lack of difference in rate of regeneration of middle and basal thirds in *T. crocea*. Differences in the growth habits of the species are in harmony with the experimental findings. In T. marina (see illustration in Child, '15, p. 90), there are no branches but the base of each stem runs along the substratum as a stolon for some distance, then turns vertically, and gives rise to a new hydranth at its tip. This method of growth indicates that the hydranth in T. marina dominates a considerable length of stem. The Atlantic coast species, on the other hand, branch freely, frequently at a relatively short distance from the terminal hydranth. This fact suggests that the dominance of the terminal hydranth does not extend very far proximally. Beyond this limit the axial differences along the stem would be slight or absent. It it probable that if shorter distal pieces of the stem of T. crocea were cut into thirds, a difference would be found in the regeneration rate of the middle and basal thirds. In both my and Banus' experiments, however, rather long stems were used.

3. Relation of Rate of Regeneration to the Length of the Piece.-In a previous paper ('20) I reported that the length of the piece has little effect on the rate of regeneration when diameter and level of the distal cut are constant, except when the pieces are very short. This result was again verified on T. crocea and on the Maine species. Thus in 16 pairs of pieces of *T. crocea*, the two of each pair being 10 and 5 mm. long, respectively, with the distal cut taken at the same level in both, the 5 mm. pieces regenerated oral hydranths first in eight cases, the 10 mm. first in eight cases. It is understood that the diameter of the apical end was the same in the two members of each pair. At Swan's Island an experiment was performed comparing the rates of regeneration of pieces 15, 10 and 5 mm. long, the distal diameter and level of the distal cut being the same in the three pieces of each set. The rate of regeneration of the 5 and 10 mm. pieces was equal throughout this experiment; and this was also the case with most of the 15 mm. pieces, but a few of them preceded in production of hydranths the shorter pieces by a short time interval.

When the pieces are shorter than 5 mm. in length the production of hydranths is greatly slowed down, so that the regeneration

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of pieces 2 mm. in length, for instance, runs far behind that of longer pieces.

4. Relation of Rate of Regeneration to the Diameter of the Stem.— It was previously reported that the time between cutting and emergence of oral hydranths is noticeably shorter the more slender the stem. This was again verified on the Maine species. It is reasonable to believe that this result is correlated with the fact, given in the first part of this paper, that the rate of oxygen consumption is higher, the less the diameter of the stem.<sup>1</sup>

5. Relation of Size of the Regenerated Oral Hydranth to the Length of the Stem .- A number of measurements were made both at Woods Hole and Swan's Island of the size of the regenerated hydranths on stems of the same distal diameter and cut at the same distal level but of different lengths. Measurements were made with an ocular micrometer, and on the fully expanded hydranth some hours after emergence. The length of the tentacles, the length of the body (distance from tip of manubrium to constriction just below base of hydranth), and width of the widest part of the hydranth (part bearing the proximal tentacles) were measured. The most extensive experiment of this kind was done at Swan's Island. A number of pieces having the same apical level but of different lengths and diameters were cut and placed in one bowl. After regeneration the dimensions of the regenerated oral hydranths and of the original piece were taken. These data are given in Table III. and are arranged with reference to the diameter of the apical end of the piece.

These data show that the dimensions of regenerated oral hydranths are about the same on pieces of quite different lengths but of the same diameter and with their apical ends at the same level. This statement would probably not apply to very short pieces (under 2 mm.) but as shown in the table pieces as short as 2 mm. may regenerate hydranths as large as those from pieces several times as long. Some of the longest pieces may produce

<sup>1</sup> Stems whose diameter is smaller are of course also smaller in other particulars total length and size of hydranths. As pointed out later they are simply younger as a rule than larger stems. It seems convenient to take the diameter of the stem as a measure of age. Wherever it is stated throughout this paper that the diameter of the piece is smaller it is to be understood that such pieces were taken from stems of general small proportions. hydranths slightly larger than much shorter pieces but this difference is slight at best and in no wise proportional to the great differences in length of such pieces.

## TABLE III.

Dimensions of Regenerated Oral Hydranths with Reference to Length of Piece.

Length of Piece, mm.	Diameter at Apical End of Piece.	Length of Body of Hydranth.	Width of Body of Hydranth.	Length of Tentacles.
2	6.0	15	6	12
5	6.0	18	8	II
9	6.0	21	IO	15
2	6.5	1.4	8	IO
5.5	6.5	20	9	12
5	6.5	15	7	I 2
15	0.5	24	II	17
15	0.5	20	8	14
25	6.8	TO	8	τ <i>ά</i>
2.3 5 5	6.8	22	0	14
3.3	6.8	20	7	10
J.J TO 5	6.8	23	12	18
10.5	6.8	23	12	15
- 1		-0		
2	7.0	20	7	16
5.5	7.0	18	8	12
5.5	7.0	19	8	13
6	7.0	19	9	14
10	7.0	22	9	14
IO	7.0	23	IO	15
IO	7.0	25	ΙI	15
10.5	7.0	19	9	14
11	7.0	18	8	I 2
5	7.2	19	8	II
15	7.2	24	10	13
_		TO	TO	TE
5	1.5	19	10	12
2	1.5	20	9	12
5.5	1.5	20	10	15
11	1.5	20	10	22
11.5	1.5	24	12	IO
10	1.3	-1	1.44	*9
2.5	8.0	21	8	15
10	8.0	25	I 2	17
2.5	8.5	22	9	II
5.5	8.5	24	I 2	I 2
5.5	8.5	22	9	I 2
10	8.5	22	II	16

Length in millimeters; other figures units of ocular micrometer.

6. Relation of Size of the Regenerated Oral Hydranth to the Diameter of the Stem .- Some experiments were performed on this matter. It was found that the size of the hydranth is slightly larger in stout than in slender stems when other factors are eliminated. This difference is detectable only when the difference in diameter of the stems is considerable. In Table III., where there are but small differences in diameter, no definite relation between dimensions of regenerated oral hydranths and diameter of the stems appears. But when stems differing markedly in diameter are compared the dimensions of the regenerated oral hydranths are seen to bear some slight relation to the diameter of the oral end of the pieces. One experiment of this kind is given in Table IV. Stout and slender stems from the same colony, of the same length, and cut at the same apical levels were allowed to regenerate and the resulting oral hydranths measured as in the preceding section. There were ten stems of each lot; these have been averaged in the table for brevity.

## TABLE IV.

DIMENSIONS OF REGENERATED ORAL HYDRANTHS WITH REFERENCE TO THE DIAMETER OF THE APICAL END OF THE ORIGINAL STEMS.

Ten pieces in each lot, pieces 20 mm. long. Figures, units of the micrometer scale.

	Diameter Apical End.	Length Body of Hydranth.	Width Body of Hydranth.	Length Tentacles.
	Lo	t of Slender Sten	ns.	
Min	5- <mark>5</mark>	19	8	I5
Aver.	7.0 6.2	20 23	II IO	21 17
	L	ot of Stout Stem	s.	
Min	7-5	2.2	1 I	18
Max	11.0 8.7	30 25	15 12	22 I 9

Table IV. shows that in the stouter stems the dimensions of the hydranth are slightly larger on the average than in the slender stems. The chief difference is in the width of the hydranth. The result is readily understandable when it is recalled that in the regeneration of Tubularia, the new hydranth is laid down in the old coenosarc. The new hydranth will then be necessarily broader the stouter the stem.

It will be perceived that the differences in dimensions of regenerated hydranths on stems of different diameter are in no wise proportional to the diameters. In Table IV., the diameters differ by 40 per cent. on the average, while the differences in dimensions of the regenerated hydranths average 10 to 20 per cent. Further, the more slender stems regenerate in a shorter length of time.

7. Relation of the Size of the Regenerated Oral Hydranth to the Level of the Stem.—In determining this matter it is necessary that the diameter of the apical ends of the pieces to be compared be the same, for, as shown above, diameter affects the dimensions of the regenerated hydranth. It is a little difficult to obtain pieces from different levels of the same apical diameter since in *Tubularia* the stem generally tapers towards the base. However, it is occasionally possible to find stretches of stem of approximately the same diameter throughout or even some which increase in diameter proximally. Only such have been used in making the comparison. From such stems apical and basal pieces of equal length were cut and after regeneration the dimensions of the regenerated oral hydranths determined. Some data of this kind, obtained at both Woods Hole and Swan's Island are given in Table V.

It is obvious to the eye and measurements also demonstrate that in pieces of the same diameter and length but taken from different levels, the dimensions of the regenerated oral hydranth are nearly always greater on the apical than on the basal piece. Level is thus the most important factor in determining the dimensions of regenerated oral hydranths. These size differences of oral hydranths also of course appear in pieces cut from the usual type of stem, where the diameter of the basal piece is smaller than that of the apical piece; and are too great to be accounted for merely on the differences in diameter.

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## TABLE V.

### DIMENSIONS OF REGENERATED ORAL HYDRANTHS ON PIECES OF THE SAME LENGTH AND DISTAL DIAMETERS BUT FROM DIFFERENT LEVELS.

Level of	Diameter	Length Body	Width Body	Length	
Pieces.	Distal End.	of Hydranth.	of Hydranth.	Tentacles.	
Apical	10.0	15	7	20	
Basal	10.0	12	7	16	
Apical	5.0	14	5	I 5	
Basal	5.0	10		7	
Apical	6.5	20	10	23	
Basal	6.5	16	7	18	
Apical	5.5	30	9	23	
Basal	5.5	17	8	27	
Apical	7.I	20	10	25	
Basal	7.I	17	9	18	
Apical	6.6	27	1 I	45	
Basal	6.8	24		45	
Apical	6.4	28	10	40	
Basal	6. <b>4</b>	23	10	35	
Apical	6.3	23	777	27	
Basal	6.3	21		12	

Figures, units of the micrometer scale.

## DISCUSSION.

The foregoing facts together with others available from the literature support the conception of the existence of a metabolic gradient along the main axis in *Tubularia* and other lower forms; and of a relation between this gradient and the rate of regeneration.

It is shown in this paper that the rate of oxygen consumption in the stem of *Tubularia* is higher in apical than in basal levels. It thus appears that there exists a gradation in respiratory rate along the stem of *Tubularia*. Similar respiratory gradients along the main axis were previously reported for other lower invertebrates: *Corymorpha*, *Grantia*, *Planaria*, several annelids (Hyman, '22, '23, '25, Hyman and Galigher, '21). It is reasonable to believe that they are of universal occurrence among at least the lower Metazoa. We believe that such differences in rate of chemical activity (with which are doubtless associated other graded differences) constitute the basis of the phenomenon of polarity.

It appears further that there exist in these organisms permanent electric currents whose direction of flow bears a definite relation to the respiratory gradient (for data on *Tubularia*, see Hyman, '20, for other hydroids, Hyman and Bellamy, '22, Lund, '22).<sup>2</sup> This relation is the following: any part of the organism is electronegative (in the external circuit) to any part having a lower respiratory rate than itself. It is probable that the gradation in rate of chemical activity is the chief cause of the electrical gradient. Some biologists are of the opinion that these electric currents constitute a tool, so to speak, which enables one part of an organism to affect another part.

There is some indication that in the hydroids the gradient is steeper in the more apical levels and gradually flattens out basally. This inference is drawn chiefly from the electrical data, the potential difference being greatest in apical levels (Hyman and Bellamy, '22, pp. 332-33, Lund, '22, p. 490); but in Corymorpha the respiratory evidence is to the same effect (Hyman, '22). Regardless of the slope of the gradient in distal levels, it appears certain from electrical and other data that the gradient is slight or absent or even reversed in the proximal levels of hydroid stems (see references just given). It follows that at a certain distance from the apical end, the primary gradation practically disappears and new gradations running in the same or the reverse direction may be initiated. The distance to which the primary gradation extends coincides with the limits of the individual and beyond this point buds, zooids, etc., may arise, if the constitution of the protoplasm permits asexual reproduction; or if asexual reproduction is impossible, the basal or caudal parts of the organism may be more or less independent physiologically or nervously of

<sup>2</sup> In a later paper ('25) Lund reversed his statement in the 1922 paper as to the direction of the current in *Obelia*, without offering any explanation of the contradiction. It may be pointed out that Hyman and Bellamy ('22) tested the P. D. along the main axis of colonies of a species of *Obelia* common at Friday Harbor and identified by Professor Nutting as *Obelia borealis* and of *Obelia geniculata* at Woods Hole; and in both species found distal levels electronegative to proximal in agreement with the statement in Lund's 1922 paper but contrary to his statement in 1925.

anterior levels. The existence of an oral or apical end inhibits the formation of any other oral or apical end within the distance limit over which the control of the former extends (see Child, '15, Chapter IV.).

Differences in rate of regeneration (time between cutting and completion of oral or apical structures) with respect to level constitute further evidence of the existence of a metabolic gradient in hydroids. The more apical the level in the whole within the limits of the primary gradient from which the piece is taken the more rapidly does it produce a new apical end. This generalization has been shown to hold in a large number of lower invertebrates, mostly collenterates, e.g.,-Eudendrium (Goldfarb, '07), Tubularia (Driesch, Morgan, Child, etc., for references see Hyman, '20), Corymorpha (Torrey, '10), Pennaria (Gast and Godlewski, '03), Obelia (Billard, '04, Lund, '23), Cerianthus (Child, '03), Planaria (Child, '11), annelids (Hyman, '16). Further proof of the correctness of this generalization with regard to *Tubularia* is presented in this paper. It is also shown that such axial differences in rate of regeneration are independent of size or mass differences at different levels. It can scarcely be doubted that the metabolic gradient is the direct or indirect cause of the apico-basal gradation in rate of regeneration of oral or apical structures.

Another instance of the dependence of regeneration rate on metabolic rate is the difference in these regards between organisms of different ages (sizes). It is shown in this paper and previously (Hyman, '20) that in *Tubularia* the rate of regeneration is more rapid the smaller the diameter of the piece; and further that the rate of oxygen consumption is higher the smaller the diameter. There is thus a correlation between rate of production of oral hydranths and rate of respiratory metabolism; and it is scarcely to be doubted that the latter is the direct cause of the former. It is probable that in *Tubularia* the dimensions of the stem (diameter, total length, size of hydranth) vary inversely with age and that we are really dealing here with age and not size differences. An inverse relation between respiratory rate and age (size) appears to be universal throughout the animal kingdom. In a previous paper (Hyman, '19) I reviewed this matter and quoted a considerable body of evidence in support of this generalization. Since then I have obtained additional evidence (unpublished) of the inverse relation between respiratory rate and size in *Corymorpha*, starfish, nudibranchs, and tadpoles; and other data have appeared in the literature (*e.g.*, Smith, '25). The relation here found in *Tubularia* between rate of regeneration and age probably also is of general application. Przibram ('07) gives a discussion of this matter and reaches the generalization that the rate of regeneration is more rapid the younger the animal and declines with age.

Not only is there a relation between rate of regeneration (time between cutting and completion of oral or apical structures) and respiratory rate but the amount of tissue regenerated in a given time appears also to be dependent upon metabolic rate. This applies both to axial differences and age differences. In general the more apical the level from which the piece is taken the larger is the size of the oral or apical end regenerated, and the greater the total mass of regenerated tissue. This result cannot be ascribed to differences in mass of pieces from different levels for it also holds when the pieces are of equal mass. It is shown in this paper that in Tubularia, the regenerated oral hydranth is larger on apical than basal pieces, when the length and apical diameters of such pieces are the same. Driesch ('99) and Child ('07) had previously noted that the length of the primordium of the oral hydranth is greater the more apical the piece. A similar relation between level and the size of regenerated apical structures or total amount of tissue regenerated was observed by Billard ('04) in Obelia, Child ('03) in Cerianthus and Planaria ('11) and Morgulis ('07) in annelids.

The amount of tissue regenerated in a given time is also greater the young (smaller) the organism, relative to its size. This is shown to be the case in *Tubularia*, pieces from smaller stems producing relatively larger hydranths in a shorter time than pieces of equal length from large stems. A similar relation between age and rate of formation of new tissue was found by Zeleny ('07), Ellis ('08), and Scott ('09).

A third factor appears to be involved as regards the amount of tissue regenerated in a given time. This factor is the degree of injury relative to the mass of the regenerating piece or organism. Some years ago under the leadership of Zeleny there was considerable interest in this matter. A number of papers dealing with this subject were published (Zeleny, '03, '05*a*, '05*b*, '07, Ellis, '07). A general agreement was reached by the workers in this field that any one part is replaced at a more rapid rate, the greater the amount of tissue removed at the original operation. It is possible that this result also depends on metabolic rate; for every wound is the locus of an increase in metabolic rate and the greater the number of wounds and the smaller the mass of tissue remaining the greater is the stimulation of respiratory metabolism not only at the wounds but also in the adjacent uninjured parts.

In general, then, it appears that the size of regenerated oral or apical structures and the amount of tissue produced in a given time are causally related to the metabolic rate of the regenerating mass.

When metabolic factors such as level, age, or wound stimulation are not involved, the mass of the piece appears to have little or no effect upon the amount of tissue regenerated in a given time. Thus it is shown in this paper for *Tubularia* that pieces differing considerably in length and hence total mass regenerate oral hydranths of equal size in equal lengths of time. Because of this lack of relation between mass and regeneration it can be stated as a generalization that the smaller the original mass (within certain limits of course) the greater relative to its mass is the amount of tissue produced in a given time. Other data in support of this statement will be found in many of the papers already cited.

It remains to consider a paper by Lund ('23) on regeneration in *Obelia*, in which paper certain conclusions are stated which seem to be at variance with those presented here. In the regeneration of *Obelia* as previously noted by Billard ('04) a process grows out from the cut surface and the hydranth differentiates at the end of this outgrowth. Lund has studied the time occupied by this growth process and the rate of elongation of the outgrowth in a series of pieces cut in apico-basal sequence from the main stem of colonies of *Obelia*. Lund finds as did Billard

('04) that the time between cutting and completion of the polyp is shorter the more apical the piece. But according to Lund the time between the beginning and end of the outgrowth is the same at all levels. By defining the regeneration period as the time period during which the outgrowth is elongating, Lund is able to reach the conclusion that the "rate of regeneration" is the same at all levels. It may merely be pointed out that adopting some particular definition in no wise alters the facts of the matter, which are the same for Obelia as for other cœlenterates. Lund is able to state that the rate of regeneration does not differ at different levels only because his definition of the expression "rate of regeneration" differs from that used by other workers. The point raised by Lund that the time interval from the beginning to the end of the elongation process is the same at all levels may be correct but it does not seem to me to be proved by his tables and graphs. However, it is difficult to come to any decision on the matter, as neither the time of beginning of growth of the apical pieces nor of completion of growth of the basal pieces is given. It is very probable, nevertheless, that the chief differences in regeneration rate at different levels lie in the early part of the regeneration period. It is admitted by Lund and is shown by his tables and graphs that the rate of elongation of the outgrowth is faster the more apical the level. Billard ('04) had previously made a similar observation; he noted that the sum of the lengths of the outgrowth at both ends of each piece is greater the more apical the piece. In the face of his own data, Lund still attempts to maintain the conclusion that the rate of elongation is the same at all levels on the assumption that the rate of elongation decreases apico-basally because the mass of the pieces decreases in the same direction. He assumes without any proof whatever that the amount of tissue regenerated is proportional to the mass of the piece. Such an assumption is incorrect. As already pointed out considerable differences in length of pieces have no effect on either the time required for regeneration or the amount of tissue produced in that time; and such slight differences in amount regenerated as are correlated with differences in diameter are counterbalanced by the more rapid rate of regeneration of pieces of smaller diameter. All of the available evidence indicates that the mass of regenerated tissue is not proportional to the original mass of the piece but to the contrary is relatively greater the smaller the latter. In view of all of the facts it is practically certain that the apico-basal sequence in rate of elongation observed in *Obelia* pieces is the result of differences in level. Even though the time occupied by the elongation process may be the same at different levels, as insisted by Lund, still the length of outgrowth produced in that time is greater the the more apical the piece; and hence the "rate of regeneration," even using this expression as defined by Lund, decreases apicobasally in *Obelia* as in other lower forms.

## SUMMARY.

1. The rate of oxygen consumption per unit volume of cœnosarc is greater in apical than in basal halves of distal regions of the stem of *Tubularia*.

2. The rate of oxygen consumption per unit volume of cœnosarc is greater the younger the stem (smaller its diameter).

3. The time between cutting and completion of oral hydranths is shorter the more apical the piece in pieces of equal length from distal levels of the stem of *Tubularia*.

4. The statement in 3 may or may not hold for proximal regions of the stem, depending on the species.

5. The time between cutting and completion of oral hydranths is independent of the length of the piece when the apical end of the pieces is taken at the same level, except in very short pieces.

6. The time between cutting and completion of oral hydranths is shorter the younger the stem (smaller the diameter).

7. The size of the regenerated oral hydranths is almost entirely independent of the length of the piece, when the apical end of the pieces is taken at the same level, except in very short pieces.

8. The size of the regenerated oral hydranth is slightly smaller especially as to width the smaller the diameter of the stem but not proportionally smaller.

9. The size of the regenerated oral hydranth is absolutely larger on apical than on basal pieces of equal mass taken from distal regions of the stem.

10. In general there is a relation between respiratory rate and

regeneration. The higher the respiratory rate the shorter is the time interval between cutting and completion of oral hydranths and the larger is the size relatively or absolutely of the regenerated oral hydranth.

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